TITLE

PURINE METABOLISM GENES IN PLANTS

This application claims the benefit of U.S. Provisional Application No. 60/146473, filed July 30, 1999.

BACKGROUND OF THE INVENTION

Purines are components of DNA and RNA. Purine triphosphates also serve as chemical energy storage media for the cell. Regulation of purine synthesis and metabolism within the cell is critical to functions of all cells. Most mutations affecting nucleotide biosynthetic enzymes are lethal, although certain redundancy and salvage pathways may moderate the deleterious effects of some of these mutations.

Adenosine deaminase and AMP deaminase are two enzymes that convert adenosine to inosine. Mutations in these genes cause disruptions in the salvage and catabolism of adenosine and AMP. In humans these mutations may lead to death of white blood cells (lymphocytes) which in turn cause severe immunodeficiencies (Wilson et al. (1991) *Science 252*:1278-1284; Morgan and Anderson (1993) *Ann Rev Biochem 62*:191-217; Markert (1994) *Immunodeficiency 5*:141-157). Selective disruption of adenosine or AMP deaminase activities may be exploited in the identification and production of novel herbicides, insecticides, and fungicides.

SUMMARY OF THE INVENTION

The present invention concerns an isolated polynucleotide comprising: (a) a first nucleotide encoding a polypeptide that is at least 35 amino acids in length and that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 12, 14, 16, 18, 20, and 22, or (b) a second nucleotide sequence that is a complement of the first nucleotide sequence.

In a second embodiment, the isolated polynucleotide of the invention comprises a nucleotide having a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 11, 13, 15, 17, 19, and 21.

In a third embodiment, this invention concerns an isolated polynucleotide comprising at least 30 (preferably at least 40, most preferably at least 60) contiguous nucleotides derived from a member selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 11, 13, 15, 17, 19, and 21, or a complement thereof.

In a fourth embodiment, this invention relates to a chimeric gene comprising an isolated polynucleotide of the present invention operably linked to at least one suitable regulatory sequence.

In a fifth embodiment, the present invention concerns an isolated host cell comprising a chimeric gene or an isolated polynucleotide of the present invention. The host cell may be

eukaryotic, such as a yeast or a plant cell, or prokaryotic, such as a bacterial cell. The present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide or a chimeric gene of the present invention.

In a sixth embodiment, the invention also relates to a process for producing an isolated host cell, the process comprising either transforming or transfecting an isolated host cell with a suitable chimeric gene or isolated polynucleotide.

In a seventh embodiment, the invention concerns an isolated polypeptide that is at least 35 amino acids in length and that has at least 85% identity based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 12, 14, 16, 18, 20, and 22.

In an eighth embodiment, the invention relates to a method of selecting an isolated polynucleotide that affects the expression level or activity of an AMP or adenosine deaminase in a host cell, preferably a plant cell. According to the invention, the method comprises: (a) constructing an isolated polynucleotide or an isolated chimeric gene of the present invention; (b) introducing the isolated polynucleotide or the isolated chimeric gene into a host cell; (c) measuring the level of the AMP or adenosine deaminase polypeptide or enzyme activity in the host cell containing the isolated polynucleotide; and (d) selecting a suitable polynucleotide via comparing the level of the AMP or adenosine deaminase polypeptide or enzyme activity in the host cell containing the isolated polynucleotide with the level of the AMP or adenosine deaminase polypeptide or enzyme activity in a host cell that does not contain the isolated polynucleotide.

In a ninth embodiment, the invention concerns a method of obtaining a nucleic acid fragment encoding a substantial portion of an AMP or adenosine deaminase polypeptide, preferably a plant AMP or adenosine deaminase polypeptide, comprising: synthesizing an oligonucleotide primer comprising at least 30 (preferably at least one of 40, most preferably at least one of 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 11, 13, 15, 17, 19, and 21, or from a complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of an AMP or adenosine deaminase amino acid sequence.

In a tenth embodiment, this invention relates to a method of obtaining a nucleic acid fragment encoding all or a substantial portion of an AMP or adenosine deaminase comprising: probing a cDNA or genomic library with a labeled, isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with the isolated polynucleotide; and isolating the identified DNA clone.

In an eleventh embodiment, this invention concerns a composition, such as a hybridization mixture, comprising an isolated polynucleotide of the present invention.

In a twelfth embodiment, this invention concerns a method for obtaining a transformed cell comprising: (a) transforming a host cell with the chimeric gene of the present invention or an expression cassette comprising an isolated polynucleotide of the present invention; and (b) growing the transformed host cell, preferably a plant cell, such as a monocot or a dicot plant cell, under conditions which allow expression of the polynucleotide in an amount sufficient to complement a null mutation.

In a thirteenth embodiment, this invention relates to a method of altering the level of expression of enzymes involved in purine metabolism in a host cell comprising: (a) transforming a host cell with a chimeric gene of the present invention; and (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene alters levels of the enzymes involved in purine metabolism in the transformed host cell.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of an enzyme involved in purine metabolism, the method comprising the steps of: (a) transforming a host cell with a chimeric gene of the invention; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the enzyme in the transformed host cell; (c) optionally purifying the expressed enzyme by the transformed host cell; (d) treating the enzyme with a compound to be tested; and (e) comparing the activity of the enzymes that has been treated to the activity of an untreated enzyme, thereby selecting compounds with inhibitory activity.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTINGS

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listings.

Figure 1 depicts the biochemical pathway leading to purine catabolism. The steps regulated by AMP deaminase and adenosine deaminase are noted.

Figure 2 shows a comparison of the amino acid sequences of corn, rice, soybean, and wheat AMP deaminases (SEQ ID NOs:14, 16, 18, and 20, respectively) and the *Arabidopsis thaliana* AMP deaminase (SEQ ID NO:23 [gi 7484807]).

Figure 3 shows a comparison of the amino acid sequence of the soybean adenosine deaminase (SEQ ID NO:12) to the *Escherichia coli* adenosine deaminase (SEQ ID NO:24 [gi 2506342]).

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a

substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1

Enzymes Involved in Purine Metabolism

AMP Deaminase	Clone Designation	SEQ ID NO: (Nucleotide)	SEQ ID NO: (Amino Acid)
Corn (*Zea mays*)	p0091.cqrav79r	1	2
Rice (*Oryza sativa*)	rsl1n.pk001.a12	3	4
Soybean (*Glycine max*)	sgs3c.pk001.m22	5	6
Wheat (*Triticum aestivum*)	wr1.pk0133.f5	7	8
Corn (*Zea mays*)	p0091.cqrav79r:fis	13	14
Rice (*Oryza sativa*)	rsl1n.pk001.a12:fis	15	16
Soybean (*Glycine max*)	sgs3c.pk001.m22:fis	17	18
Wheat (*Triticum aestivum*)	wr1.pk0133.f5:fis	19	20
Adenosine Deaminase	**Clone Designation**	**SEQ ID NO: (Nucleotide)**	**SEQ ID NO: (Amino Acid)**
Corn (*Zea mays*)	p0005.cbmfm83r p0005.cbmfm83rb	9	10
Soybean (*Glycine max*)	sls2c.pk003.f5	11	12
Soybean (*Glycine max*)	sls2c.pk003.f5	21	22

The Sequence Listing contains the standard one-letter codes for nucleotides and the three-letter codes for amino acids under the IUPAC-IUBMB (*Nucleic Acids Res. 13*:3021-3030 (1985); *Biochemical J. 219 (No. 2)*:345-373 (1984)). The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822. The information disclosed in SEQ ID NOs:1-12 is contained in U.S. Provisional Application No. 60/146473, filed July 30, 1999.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, the terms "polynucleotide," "polynucleotide sequence," "nucleic acid sequence," and "nucleic acid fragment" or "isolated nucleic acid fragment" are used interchangeably. A polynucleotide may be a RNA or DNA that may be single- or double-stranded, and may contain synthetic, non-natural or altered nucleotide bases. A DNA polynucleotide may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least one of 30 contiguous nucleotides, preferably at least one of 40 contiguous nucleotides, most preferably one of at least 60 contiguous nucleotides

derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, or the complement of such sequences.

The term "isolated" polynucleotide or polypeptide refers to a molecule that is substantially free from other cellular molecules, such as other chromosomal and extrachromosomal DNA and RNA or other proteins, that normally accompany or interact with it as found in its naturally occurring environment. Isolated polynucleotides or polypeptides may be purified from a host cell in which they naturally occur. Conventional nucleic acid or protein purification methods are well known to skilled artisans. The term also embraces recombinant and chemically synthesized polynucleotides or polypeptides.

The term "recombinant" means, for example, that a nucleic acid sequence is made, or modified, by an artificial combination of two or more otherwise separated nucleic acid fragments by chemical synthesis or by genetic engineering techniques.

As used herein, "contig" refers to a contiguous nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share significant common or overlapping regions of sequence homology and can be assembled into a single contiguous nucleotide sequence, to form a "contig". Methods for comparing and

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment, or its transcripts, to alter gene expression patterns or levels, for example via gene silencing through antisense or co-suppression.

Substantially similar nucleic acid fragments may be obtained by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell.

A substantially similar nucleic acid fragment representing at least 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using substantially similar nucleic acid fragments,

that is, nucleic acid molecules representing less than the entire coding region of a gene, or having less than 100% sequence identity with the gene to be suppressed.

Substantially similar polypeptides refer to polypeptides having one or more amino acid substitutions, additions or deletions that do not significantly affect the function of the polypeptide. One skilled in the art recognizes that conservative substitutions, whereby a residue is substituted by another with like characteristics, result in polypeptides with substantially similar functions. Typical such substitutions are among Ala, Val, Leu and Ile; between Ser and Thr; between Asp and Glu; between Asn and Gln; and between Lys and Arg; or between Phe and Tyr. Substantially similar polypeptides may have several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids substituted, deleted, or added in any combination.

Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product.

Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least 30 (preferably at least one of 40, most preferably at least one of 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of an AMP or adenosine deaminase.

It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof. The terms "substantially similar" and "corresponding substantially" are used interchangeably herein.

A method of selecting an isolated polynucleotide that affects the level of expression of a gene in a virus or in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial) may comprise: constructing an isolated polynucleotide of the present invention or an chimeric gene of the present invention; introducing the isolated polynucleotide or the isolated chimeric gene into a host cell that expresses a native equivalent of the isolated polynucleotide; measuring the level of expression of the native gene in the host cell; and comparing the level of a polypeptide or enzyme activity in the host cell containing the

isolated polynucleotide with the level of a polypeptide or enzyme activity in a host cell that does not contain the isolated polynucleotide.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode, as determined by algorithms commonly employed by those skilled in this art. Suitable isolated polynucleotides of the present invention encode polypeptides that are at least about 70% identical, preferably at least about 80%, more preferably at least about 85%, still more preferably at least about 90%, and most preferably at least about 95% identical to the amino acid sequences reported herein.

Suitable nucleic acid fragments not only have the above identities but typically encode a polypeptide having at least 10, preferably 20, more preferably 30, still more preferably 50, more preferably at least 100, more preferably at least 150, still more preferably at least 200, and most preferably at least 250 amino acids.

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

Amino acid and nucleotide sequences can also be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools

that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers.

Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence.

The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of these sequences, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, it is contemplated that the instant invention encompasses complete sequences of the full-length molecules comprising the sequences as reported in the accompanying Sequence Listing, as well as substantial portions thereof.

"Codon degeneracy" refers to divergence in the genetic code permitting variations of the nucleotide sequence without affecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein.

The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to code for a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable, and within the ordinary skill of the skilled artisan, to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building blocks, which may be chemically synthesized, using procedures known to those skilled in the art. These building blocks are ligated or annealed to form larger nucleic acid fragments that may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized," as related to a nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid

fragments may be accomplished using well-established procedures. Automated chemical synthesis can be performed using one of a number of commercially available machines.

Chemical synthesis of polynucleotides allows the tailoring of a polynucleotide for optimal gene expression based on optimization of the nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates that successful gene expression is more likely if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that code for a polypeptide molecule. A gene may also encode an RNA molecule. As generally understood, a gene includes regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence, as well as intervening sequences (introns) between individual coding segments (exons). "Native gene" refers to a gene with its native regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Indigenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign-gene" refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes may be native genes inserted into a non-native organism, or they may be chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence that can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or may be composed of different elements derived from different promoters found in nature, or may

even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters." New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants 15*:1-82. **(Please use a more recent reference)** It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

"Translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol. 3*:225-236).

The term "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and includes polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or other aspects of gene expression. The polyadenylation signal is usually characterized by effecting the addition of a polyadenylate homopolymer to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell 1*:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript. When the primary transcript has undergone posttranscriptional processing, it becomes a mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into polypeptides by the cell. "cDNA" refers to DNA that is complementary to and derived from an mRNA template. The cDNA can be single-stranded or converted to double stranded form using, for example, the Klenow fragment of DNA polymerase I. "Sense-RNA" refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that may block the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the target gene, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence.

"Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments to form a single polynucleotide, so that the function of one fragment is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation. For example, a coding region could be linked to a promoter such that an sense or an antisense transcript is expressed. In addition, some regulatory elements can function in a orientation-independent manner.

The term "expression," as used herein, refers to the transcription of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide.

"Antisense inhibition" refers to the production of antisense RNA transcripts, which in turn suppress the expression of the target gene. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts, which suppress the expression of identical or substantially similar foreign or indigenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

"Altered levels" of expression, or "altered expression," refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Null mutant," when used to refer to a cell, means that the cell either lacks the expression of a certain gene product or expresses a gene product which is inactive or does not have any detectable expected function.

"Mature protein" or the term "mature" when used in describing a protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed (need for functional reference?). "Precursor protein" or the term "precursor" when used in describing a protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in a plant cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53).

If the protein is to be directed to a vacuole, a vacuolar targeting signal (has not appeared previously) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Physiol. 100*:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, preferably resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of plant transformation methods include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol. 143*:277) (any newer reference?) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature 327*:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Thus, isolated polynucleotides of the present invention can be incorporated into recombinant constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., Cloning Vectors: A Laboratory Manual, 1985, supp. 1987; Weissbach and Weissbach, Methods for Plant Molecular Biology, Academic Press, 1989; and Flevin et al., Plant Molecular Biology Manual, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

"PCR" or "polymerase chain reaction" is well known by those skilled in the art as a technique used for the amplification of specific DNA segments (U.S. Patent Nos. 4,683,195 and 4,800,159).

The present invention concerns an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) first nucleotide sequence encoding a polypeptide of at least 30, preferably 60, still preferably 100, more preferably 150, still more

preferably 200, more preferably 250, more preferably 300, still preferably 350, still more preferably 400, still preferably 450, more preferably 500, more preferably 550, and most preferably 650 amino acids, having at least at least 30%, preferably 35%, still preferably 40%, more preferably 45%, still more preferably 50%, more preferably 55%, more preferably 60%, still preferably 65%, still more preferably 70%, still preferably 75%, more preferably 80%, more preferably 85%, and most preferably 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, or (b) a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Preferably, the first nucleotide sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21. A particularly preferred polypeptide of the invention has an amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, or 22.

This invention provides nucleic acid fragments encoding at least a portion of several AMP or adenosine deaminases. These nucleic acids have been isolated and identified by comparison of plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art.

The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction and ligase chain reaction).

For example, genes encoding AMP or adenosine deaminases, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, an entire sequence can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. For example, one primer may be based on the sequence of the instantly disclosed polynucleotide molecules, the other primer may be based on polyA or the sequence on a vector, depending on the template used in the amplification.

Specifically, PCR may be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA 85*:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (Gibco BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA 86*:5673-5677; Loh et al. (1989) *Science 243*:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques 1*:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 30 (preferably one of at least 40, most preferably one of at least 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, or the complement of such nucleotide sequences, may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

The present invention further relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a plant AMP or adenosine deaminases, comprising the steps of: synthesizing an first oligonucleotide primer comprising a nucleotide sequence of at least one of 30 (preferably at least one of 40, most preferably at least one of 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, or the complement of such nucleotide sequences; synthesizing a suitable second oligonucleotide primer, and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of an AMP or adenosine deaminase.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing the entirety of portions of the instant amino acid sequences are synthesized. These peptides are

used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences of the synthetic peptides. These antibodies can then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol. 36*:1-34; Maniatis).

In another embodiment, this invention concerns viruses and host cells comprising either the chimeric genes of the invention or an isolated polynucleotide of the invention. Examples of host cells which can be used to practice the invention include, but are not limited to, yeast cells, bacterial cells, and plant cells. Examples of suitable viruses include (need list for written description).

As was noted above, the nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are overexpressed or their expression suppressed. This would alter the level of floral gene expression in those cells and/or flower development of transgenic plants comprising these polynucleotides.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. The chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes, and 3' non-coding sequences encoding transcription termination signals. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant isolated polynucleotide (or chimeric gene) may be constructed. The skilled artisan readily recognizes that the choice of plasmid vector is dependent upon many factors, such as when the vector for protein expression, gene over-expression or suppression, in what type of host cell the vectors are propagated, and on the method that will be used to transform host plants.

The skilled artisan is well aware of the elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene.

The skilled artisan also recognizes that different transformation events may result in different levels and patterns of expression (Jones et al. (1985) *EMBO J. 4*:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics 218*:78-86), and that one may have to screen multiple transformation events to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

It may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate their secretion from the cell. Accordingly, the chimeric gene of the invention

may be further supplemented with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell 56*:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.100*:1627-1632) with or without removing targeting sequences that are already present. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of use may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants via co-suppression of antisense suppression.. A chimeric gene can be constructed by linking a gene or gene fragment encoding the polypeptide of interest in frame, either in the sense orientation or in the antisense orientation to suitable plant promoter sequences. The chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would most likely act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity, these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a plant breeding perspective. In addition, the ability to restrict the expression of a specific phenotype to a specific plant tissue using tissue-specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing appropriate regulatory elements known to the skilled artisan. Once transgenic plants are obtained, it will be necessary to screen individual transgenic plants for those that most effectively display the desired phenotype. The appropriate screening method will generally be chosen on practical grounds and are within the skills of the ordinary artisan. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or by assaying the relevant enzyme activity. A preferred method allows large numbers of samples to be processed rapidly.

In another embodiment, the present invention concerns a polypeptide of at least 35 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in microbes, and is used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* or *in vitro*. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art and may be used for the instant invention. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded floral development proteins. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 7).

Additionally, the instant polypeptides can be used to design or identify herbicides that inhibit their enzyme activities. This is desirable because inhibition of the activity of one or more of the enzymes described herein could lead to inhibition of plant growth or even plant death.

All or a substantial portion of the polynucleotides of the instant invention may also be used as probes for genetically and physically mapping (*see* Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, (1996) Academic Press, pp. 319-346, and references cited therein) the genes that they compose, and used as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes.

For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics 1*:174-181) in order to construct a genetic map.

In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individual plants representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is used to calculate the position of the instant nucleic acid sequence in a genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet. 32*:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in, for example, Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* *4*:37-41. F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; *see* Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet.* *7*:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Res.* *5*:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods for genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* *11*:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* *16*:325-332), allele-specific ligation (Landegren et al. (1988) *Science* *241*:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* *18*:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet.* *7*:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res.* *17*:6795-6807).

For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a plant population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA* *86*:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* *92*:8149-8153; Bensen et al. (1995) *Plant Cell* *7*:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see

Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

EXAMPLES

The present invention is further defined in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn, rice, soybean, and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Corn, Rice, Soybean, and Wheat

Library	Tissue	Clone
p0097	V9 7cm whorl section + ECB1, screened 1 B73+ECB1: 7-cm whorl section Growth conditions: field plots; these plants have been infested with ECB four times prior to harvest. Growth stage: unknown; V9 or V10	p0097.cqrav79r p0097.cqrav79r:fis
rsl1n*	Rice (Oryza sativa, YM) 15 day old seedling normalized	rsl1n.pk001.a12 rsl1n.pk001.a12:fis
sgs3c	Soybean Seeds 25 Hours After Germination	sgs3c.pk001.m22 sgs3c.pk001.m22:fis
wr1	Wheat Root From 7 Day Old Seedling	wr1.pk0133.f5 wr1.pk0133.f5:fis
p0005	Immature Ear, two	p0005.cbmfm83r p0005.cbmfm83rb
sls2c	Soybean (Glycine max L., Manta) infected with *Sclerotinia sclerotiorum* mycelium.	sls2c.pk003.f5 sls2c.pk003.f5:fis

*This library was normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science 252*:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

Full-insert sequence (FIS) data is generated utilizing a modified transposition protocol. Clones identified for FIS are recovered from archived glycerol stocks as single colonies, and plasmid DNAs are isolated via alkaline lysis. Isolated DNA templates are reacted with vector primed M13 forward and reverse oligonucleotides in a PCR-based sequencing reaction and loaded onto automated sequencers. Confirmation of clone identification is

performed by sequence alignment to the original EST sequence from which the FIS request is made.

Confirmed templates are transposed via the Primer Island transposition kit (PE Applied Biosystems, Foster City, CA) which is based upon the *Saccharomyces cerevisiae* Ty1 transposable element (Devine and Boeke (1994) *Nucleic Acids Res. 22*:3765-3772). The *in vitro* transposition system places unique binding sites randomly throughout a population of large DNA molecules. The transposed DNA is then used to transform DH10B electro-competent cells (Gibco BRL/Life Technologies, Rockville, MD) via electroporation. The transposable element contains an additional selectable marker (named DHFR; Fling and Richards (1983) *Nucleic Acids Res. 11*:5147-5158), allowing for dual selection on agar plates of only those subclones containing the integrated transposon. Multiple subclones are randomly selected from each transposition reaction, plasmid DNAs are prepared via alkaline lysis, and templates are sequenced (ABI Prism dye-terminator ReadyReaction mix) outward from the transposition event site, utilizing unique primers specific to the binding sites within the transposon.

Sequence data is collected (ABI Prism Collections) and assembled using Phred/Phrap (P. Green, University of Washington, Seattle). Phred/Phrap is a public domain software program which re-reads the ABI sequence data, re-calls the bases, assigns quality values, and writes the base calls and quality values into editable output files. The Phrap sequence assembly program uses these quality values to increase the accuracy of the assembled sequence contigs. Assemblies are viewed by the Consed sequence editor (D. Gordon, University of Washington, Seattle).

EXAMPLE 2

Identification of cDNA Clones

cDNA clones encoding AMP or adenosine deaminases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet. 3*:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a

sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

ESTs submitted for analysis are compared to the GenBank database as described above. ESTs that contain sequences more 5- or 3-prime can be found by using the BLASTn algorithm (Altschul et al (1997) *Nucleic Acids Res. 25*:3389-3402.) against the Du Pont proprietary database comparing nucleotide sequences that share common or overlapping regions of sequence homology. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences can be assembled into a single contiguous nucleotide sequence, thus extending the original fragment in either the 5 or 3 prime direction. Once the most 5-prime EST is identified, its complete sequence can be determined by Full Insert Sequencing as described in Example 1. Homologous genes belonging to different species can be found by comparing the amino acid sequence of a known gene (from either a proprietary source or a public database) against an EST database using the tBLASTn algorithm. The tBLASTn algorithm searches an amino acid query against a nucleotide database that is translated in all 6 reading frames. This search allows for differences in nucleotide codon usage between different species, and for codon degeneracy.

EXAMPLE 3

Characterization of cDNA Clones Encoding AMP Deaminase

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to AMP deaminase from human [*Homo sapiens*](NCBI General Identifier No. gi644509) and yeast [*Saccharomyces cerevisiae*](NCBI General Identifier No. gi351916). Shown in Table 3 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), the sequences of contigs assembled from two or more ESTs ("Contig"), sequences of contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding an entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 3

BLAST Results for Sequences Encoding Polypeptides Homologous to AMP Deaminase

		BLAST pLog Score	
Clone	Status	gi644509	gi1351916
p0097.cqrav79r	EST	179.00	
rsl1n.pk001.a12	EST	175.00	
sgs3c.pk001.m22	EST		39.3
wr1.pk0133.f5	EST		22.4

The sequence of the entire cDNA insert in the clones listed in Table 3 was determined. The BLASTX search using the EST sequences from clones listed in Table 4 revealed similarity of the polypeptides encoded by the cDNAs to AMP deaminase from *Arabidopsis* [*Arabidopsis thaliana*](NCBI General Identifier No. gi 7484807). Shown in Table 4 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), sequences of contigs assembled from two or more ESTs ("Contig"), sequences of contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 4

BLAST Results for Sequences Encoding Polypeptides Homologous to AMP Deaminase

Clone	Status	BLAST pLog Score gi7484807
p0097.cqrav79r:fis	CGS	254.00
rsl1n.pk001.a12:fis	CGS	254.00
sgs3c.pk001.m22:fis	CGS	254.00
wr1.pk0133.f5:fis	FIS	254.00

Figure 2 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:14, 16, 18 and 20, and the *Arabidopsis thaliana* sequence (SEQ ID NO:23). The data in Table 5 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 14, 16, 18 and 20, and the human (NCBI General Identifier No. gi 644509), yeast (NCBI General Identifier No. gi 351916), and *Arabidopsis thaliana* sequences (SEQ ID NO:23).

TABLE 5

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to AMP Deaminase

SEQ ID NO.	Percent Identity to gi 644509	gi 1351916	gi7484807
2	42.3%		
4	60.7%		
6		70.7%	
8		43.9%	
14			81.7%
16			82.3%
18			78.0%
20			82.6%

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of an AMP deaminase. These sequences represent the first corn, rice, soybean, and wheat sequences encoding AMP deaminase known to Applicant.

EXAMPLE 4

Characterization of cDNA Clones Encoding Adenosine Deaminase

The BLASTX search using the EST sequences from clones listed in Table 6 revealed similarity of the polypeptides encoded by the cDNAs to adenosine deaminase from *Arabidopsis* [*Arabidopsis thaliana*] (NCBI General Identifier No. gi 4115949) and yeast (*Saccharomyces cerevisiae*) (NCBI General Identifier No. gi 1703166). Shown in Table 6 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), the sequences of contigs assembled from two or more ESTs ("Contig"), sequences of contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding an entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 6

BLAST Results for Sequences Encoding Polypeptides Homologous to Adenosine Deaminase

Clone	Status	BLAST pLog Score gi 4115949	gi 1703166
Contig of p0005.cbmfm83r p0005.cbmfm83rb	Contig	14.7	
sls2c.pk003.f5	EST		17.3

The sequence of the entire cDNA insert in the clones listed in Table 6 was determined. The BLASTX search using the CGS sequence from the soybean clone listed in Table 7 revealed similarity of the polypeptide encoded by the cDNA to adenosine deaminase from *Escherichia coli* (NCBI General Identifier No. gi 2506342). Shown in Table 7 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), sequences of contigs assembled from two or more ESTs ("Contig"), sequences of contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 7

BLAST Results for Sequences Encoding Polypeptides Homologous to Adenosine Deaminase

Clone	Status	BLAST pLog Score 2506342
sls2c.pk003.f5:fis	GCS	31.52

Figure 3 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:22 and the *Escherichia coli* sequence (SEQ ID NO:24). The data in Table 8 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:10, 12, and 22, and the *Arabidopsis* (NCBI General Identifier No. gi 4115949), yeast (NCBI General Identifier No. gi 1703166), and *Escherichia coli* sequences (SEQ ID NO:24).

TABLE 8

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Adenosine Deaminase

	Percent Identity to		
SEQ ID NO.	gi 4115949	gi 1703166	gi 2506342
10	71.7%		
12		67.6%	
22			25.8%

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of an adenosine deaminase. These sequences represent the first soybean sequence encoding adenosine deaminase known to Applicant.

EXAMPLE 5

Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli*

XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst AG, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μL of ethanol. An aliquot (5 μL) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad

Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing gluphosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology 8*:833-839).

EXAMPLE 6

Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem. 261*:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) *327*:70-73, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature 313*:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al.(1983) *Gene 25*:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µl spermidine (0.1 M), and 50 µL $CaCl_2$ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µL 70% ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five µL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the

retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 7

Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene 56*:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% low melting agarose gel. Buffer and agarose contain 10 μg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies, Madison, WI) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 μL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs (NEB), Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 μg/mL ampicillin. Transformants containing the gene

encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol. 189*:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

EXAMPLE 8

Evaluating Compounds for Their Ability to Inhibit the Activity of Enzymes Involved in Purine Metabolism

The polypeptides described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 7, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant polypeptides may be expressed either as mature forms of the proteins as observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("$(His)_6$"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzyme. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the enzyme.

Purification of the instant polypeptides, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the

instant polypeptides are expressed as fusion proteins, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant polypeptides may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a $(His)_6$ peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include β–mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.

Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the instant polypeptides disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for AMP deaminase are presented by Meyers et al. (1989) *Biochem 28*:8734-8743. Assays for adenosine deaminase are presented by Pelcher in U.S. Patent No. 5,474,929.